## 8.1. Recommendations for single epidemiological studies:

**a) Study design** (including confounding)

1) The diverse epidemiological study designs differ in their potential biases. Since prospective epidemiological designs provide stronger evidence for causal inference, these studies are encouraged over the other designs for pesticide risk assessment.

2) Future epidemiological studies should be conducted using the appropriate sample size in order to properly answer the question under investigation.

3) Future studies should take into consideration heterogeneity, subpopulations, exposure windows and susceptibility periods and conditions (pregnancy, development, diseases, etc.).

4) A wide range of potential confounding variables (including co-exposure to other chemicals, lifestyle, socioeconomic factors, etc.) should be measured or accounted for during the design stage (matching) of the study.

5) Consideration of host factors that may influence toxicity and act as effect modifiers (e.g., biomarkers of susceptibility). These will include genetic polymorphisms data, such as paraoxonase-1 type.

6) Collaboration between researchers is encouraged to build-up consortia that enhance the effectiveness of individual cohorts.

7) Collection and appropriately storage of relevant biological material should be undertaken for future exposure assessment, including the use of novel technologies.

**b) Exposure** (measurement, data transformation for reporting and statistical analysis):

1) Collection of specific information on exposure should avoid as far as possible broad definitions of exposure, non-specific pesticide descriptions and broad exposures classifications such as "never" *vs.* "ever " categories. Nevertheless, these categories may be valuable under certain circumstances, e.g. to anticipate a class effect.

2) Studies which only look at broad classes of pesticides (generic groups of unrelated substances), or "insecticides", "herbicides", etc. or even just "pesticides" in general are of much less use (and may even be pretty close to useless) for risk assessment. Studies that investigate specific named pesticides and co-formulants are more useful for risk assessment.

3) Pesticides belonging to the same chemical class or eliciting the same mode of action or toxicological effects might be grouped in the same category. Further refinement with information on frequency, duration and intensity of exposure might help in estimating exposure patterns.

4) In occupational epidemiology studies, operator and worker behaviour and proper use of personal protective equipment (PPE) should be adequately reported as these exposure modifiers may significantly change exposures and thereby potential associations.

5) Indirect measures of environmental exposure for wider populations, including records on pesticide use, registry data, GIS, geographical mapping, etc. as well as data derived from large databases (including administrative databases) may be valuable for exploratory studies. If these data are not available, records/registries should be initiated. Likewise, estimation of dietary exposure to pesticide from food consumption databases and levels of pesticide residues from monitoring programs can be used as well. As with direct exposure assessment, each method of indirect measurement should be reviewed for risk of bias and misclassification and weighted appropriately.

6) Whenever possible, exposure assessment to pesticides should use direct measurements of exposure in order to establish different levels of exposure (e.g., personal exposure

metering/biological monitoring). New studies should explore novel ways of personal exposure monitoring.

7) For quantitative risk assessment, there is a need to identify exposures to named pesticides and to categorise (or better yet quantify) exposure levels. Quantitative data on exposure to a single pesticide can be provided by using human biomonitoring methods and expressing results with standardized units to normalize exposure across populations.

8) The use of the exposome concept and metabolomics in particular hold great promise for next-generation epidemiological studies both for better exposure measurement (biomarkers of exposure) for identification of vulnerable subpopulations and for biological interpretation of toxicity pathways (biomarkers of disease).

9) Improved knowledge on exposure (and toxicity) to pesticide mixtures will be beneficial for comprehensive risk assessment. Consideration of the joint action of combined exposures to multiple pesticides acting on common targets, or eliciting similar adverse effects, is relevant for risk assessment. This requires all the components of the mixture to be known as well as an understanding of the mode of action, dose-response characteristics and potential interactions between components. Characterisation of the exposure is a key element for combined exposure to multiple pesticides where the pattern and magnitude of exposure changes over time.

**c) Adverse Outcomes** (measurement, data transformation for reporting and statistical analysis):

1) Outcomes under study should be well defined and surrogate endpoints should be avoided unless they have been validated. Care must be taken when definitions of diseases and subclasses of diseases change over time, particularly for long latency diseases (cancer, neurodegenerative disorders, etc.).

2) Use should be made of biological markers of early biological effect to improve the understanding of the pathogenesis of diseases. These quantitative biological parameters from mechanistic toxicology will enhance the usefulness of epidemiology because they improve the study sensitivity, reduce misclassification and enhance human relevance as compared to findings from studies in experimental animals. Since these refined endpoints are early events in the toxicodynamic pathway and often measured on a continuous scale, they might be preferable to more overt and traditional outcomes.

3) The use of biomarkers of effect may be helpful in assessing aggregate exposure to pesticides and informing cumulative risk assessment.

4) Developing read across methods allowing health outcomes to be identified using epidemiological studies and to link acute and chronic incidents records with experimental findings.

**d) Statistical** (descriptive statistics, modelling of exposure-effect relationship):

1) Statistical analysis should be based on a priori defined analytical (statistical) protocols, to avoid *post hoc* analyses for exploratory studies and report all the results, regardless of whether they are statistically significant or not.

2) Confounding should be controlled for using appropriate statistical methods that include sensitivity analysis.

3) Data should be reported in such a way that permit, where appropriate, mathematical modelling to estimate individual/population exposures and dose-response assessment irrespective of whether direct or indirect measures are used.

4) Reports should include both unadjusted and adjusted proportions and rates of outcome of interest across studies that are based on underlying populations with different structure of relevant factors and exposures.

5) When the association between a given pesticide exposure and a disease is found to be statistically significant, particularly in (presumed) low powered studies, it would be general good practice to perform a power analysis to determine the degree to which the statistically-significant effect size estimate (e.g., OR or RR) may be artificially inflated or magnified[18].

**e) Reporting of results:**

1) These should follow practices of good reporting of epidemiological research outlined in the STROBE statement and in the EFSA guideline on statistical reporting (2014) and include the further suggestions identified in this Opinion including effect size inflation estimates.

2) Although some epidemiological research will remain exploratory and *post hoc* in nature, this should be acknowledged and supported by appropriate statistical analysis.

3) Epidemiology studies are encouraged to provide access to raw data for further investigations and to deposit their full results and scripts or software packages used for analyses.

4) Report, or deposit using online sources, all results along with scripts and statistical tools used to allow the reproducibility of results to be tested.

5) Report all sources of funding and adequately report financial and other potential conflicts of interest.

As a general recommendation, the PPR Panel encourages development of guidance for epidemiological research in order to increase its value, transparency and accountability[19]. An increased quality of epidemiological studies, together with responsible research conduct and scientific integrity, will benefit the incorporation of these studies into risk assessment.

## 8.2. Surveillance

1) Increase the reporting of acute and chronic incidents by setting up post marketing surveillance programmes (occupational and general population) as required by article 7 of EU directive 2009/128; this should be fulfilled by developing surveillance networks with occupational health physicians and by boosting the collaboration between national authorities dealing with PPP and poison control information centres.

2) Develop a valid method for assessing the weight/strength of the causal relationship ("imputability") for acute and chronic incidents, and develop glossaries and a thesaurus to support harmonized reporting between EU member states.

3) Harmonised data from member states should be gathered at the EU level and examined periodically by the Commission/EFSA and a report should be released focussing on the most relevant findings.

4) Develop an EU-wide vigilance framework for pesticides.

5) There is scope for training improvements regarding pesticide toxidromes in toxicology courses for medical and paramedical staff responsible for diagnostic decisions, data entry and management.

[18] Additional information on power and sample size recommendations and related issues including effect size magnification are provided in Annex B to this report. Specifically, a power calculation requires 3 values to be clearly reported by epidemiological studies: i) the number of subjects in the non-exposed group (including diseased and non-diseased individuals); ii) the number of subjects in the exposed group (including diseased and non-diseased individuals); and iii) the number of diseased subjects in the non-exposed group.

[19] An example is the guideline developed by the Dutch Society for Epidemiology on responsible epidemiologic Research Practice (2017).

## 8.3. Meta-analysis of multiple epidemiological studies

1) For every evidence synthesis effort, studies should be reviewed using relevant risk of bias tools. Studies with different designs, or with different design features, may require (some) different questions for risk of bias assessments.

2) Evidence syntheses should not be restricted to specific time frames; they should include the totality of evidence. These efforts are more relevant if focused on specific disease outcome or disease categories.

3) In evidence synthesis effort, beyond the quantitative synthesis of the effect sizes, there should be consideration on the calculated predictive intervals, small study effects and asymmetry bias, conflicts of interest, confounding, excess significance bias, and heterogeneity estimates.

4) In the presence of heterogeneity, studies with highly selected populations, albeit unrepresentative of their respective populations, may prove valuable and deserve consideration as they may represent genuine and not statistical heterogeneity.

5) Evidence from epidemiological studies might be pooled by taking into account a thorough evaluation of the methods and biases of individual studies, an assessment of the degree of heterogeneity among studies, development of explanations underlying any heterogeneity and a quantitative summary of the evidence (provided that it is consistent).

6) Where quantitative data of individual pesticides are available from epidemiological studies, they can be combined or pooled for dose-response modelling, which could enable development of quantitative risk estimates and points of departure (BMDL, NOAEL).

7) International consortium of cohort studies should be encouraged to support data pooling to study disease-exposure associations that individual cohorts do not have sufficient statistical power to study (e.g., AGRICOH).

## 8.4. Integration of epidemiological evidence with other sources of information

1) All lines of evidence (epidemiology, animal, *in vitro* data) should be equally scrutinised for biases.

2) Validated and harmonised methods should be developed to combine observational studies, animal/basic science studies and other sources of evidence for risk assessment.

3) Experimental and human data should both contribute to hazard identification and to dose-response assessment.

4) Epidemiological findings should be integrated with other sources of information (data from experimental toxicology, mechanism of action/AOP) by using a weight of evidence approach. An integrated and harmonized approach should be developed by bringing together animal, mechanistic and human data in an overall WoE framework in a systematic and consistent manner.

5) The AOP framework offers a structured platform for the integration of various kinds of research results.

6) Animal, *in vitro* data and human data could be assessed as a whole for each endpoint. A conclusion can be drawn as to whether the results from the experiments are confirmed by human data for each endpoint and this could be included in the Renewal Assessment Reports (RAR).

# 9. Conclusions

This Scientific Opinion is intended to help the peer review process during the renewal of pesticides authorization (and, where possible, during the approval process) under Regulation 1107/2009 which requires a search of the scientific peer-reviewed open literature, including existing epidemiological studies. These are more suitable for the renewal process of active substances, also in compliance with Regulation 1141/2010, which indicates that the dossiers submitted for renewal should include new data relevant to the active substance.

The four key elements of the terms of reference are repeated below and the parts of the text addressing the individual terms are identified in order. As they follow from the text passages grouped with each of the ToRs the recommendations relevant to each of the ToRs are also indicated as follows.

"The PPR Panel will discuss the associations between pesticide exposure and human health effects observed in the External scientific report (Ntzani et al., 2013) and how these findings could be interpreted in a regulatory pesticide risk assessment context. Hence, the PPR Panel will systematically assess the epidemiological studies collected in the report by addressing major data gaps and limitations of the studies and provide recommendations thereof".

"The PPR Panel will specifically "

1. Collect and review all sources of gaps and limitations, based on (but not necessarily limited to) those identified in the External Scientific report in regard to the quality and relevance of the available epidemiological studies. Responses in Section 3 pp 22-26, Section 5.2 pp 36-38: no Recommendations appropriate.

2. Based on the gaps and limitations identified in point 1, propose potential refinements for future epidemiological studies to increase the quality, relevance and reliability of the findings and how they may impact pesticide risk assessment. This may include study design, exposure assessment, data quality and access, diagnostic classification of health outcomes, and statistical analysis. Responses in Section 4 pp 26-35: Recommendations Section 8.1, 8.2 and 8.3 pp 57-60.

3. Identify areas in which information and/or criteria are insufficient or lacking and propose recommendations for how to conduct pesticide epidemiological studies in order to improve and optimize the application in risk assessment. These recommendations should include harmonisation of exposure assessment (including use of biomonitoring data), vulnerable population sub-groups and/or health outcomes of interest (at biochemical, functional, morphological and clinical level) based on the gaps and limitations identified in point 1. Responses in Section 4.2-4.5 pp 30-35, Section 5.3 pp 38-39. Recommendations in Section 8.1 c) 1-4.

4. Discuss how to make appropriate use of epidemiological findings in risk assessment of pesticides during the peer review process of draft assessment reports, e.g. weight-of-evidence as well as integrating the epidemiological information with data from experimental toxicology, adverse outcome pathways, mechanism of actions, etc. Responses in Section 6.2 and 6.3 pp 40-48 & 7 pp 49-56: Responses in Section 8.4 pp 60-61.

As explained above, appropriate epidemiological data and post approval surveillance may usefully contribute to the risk assessment framework by hazard identification, and - with methodological improvements - hazard characterisation. It can be improved by contributions from Weight of Evidence analysis, Uncertainty analysis, and identification and estimation of biases. It is the responsibility of applicants to collect the available relevant literature, to consider its relevance and quality using relevant EFSA criteria including those for systematic review and to introduce discussion of the outcomes within the DAR, RAR and post approval frameworks that are prescribed under EU law.

The definition of appropriate quality will require analysis of sample size, statistical procedures, estimates of effect size inflation, assessment of biases and their contribution to the conclusions drawn.

The nature of the studies will require consideration at all relevant points in the risk assessment process so that for example epidemiological data on reproductive topics will be considered alongside